IMMUNE DYSFUNCTION IN PERIPARTURIENT DAIRY COWS: EVIDENCE, CAUSES, AND RAMIFICATIONS

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INTRODUCTION

Dairy cattle are susceptible to increased incidence and severity of disease during the periparturient period and health disorders occurring during this time are especially problematic because they greatly impact the productive efficiency of cows in the ensuing lactation (Ingvartsen, 2006, Pinedo et al., 2010). Indeed, about 75% of disease incidence within herds occurs within the first month of lactation including disorders of economic significance such as mastitis, metritis, ketosis and displaced abomasum (LeBlanc et al., 2006). As such, there have been numerous studies to better understand the underlying causes of both metabolic and infectious diseases around the time of parturition in order to design more effective management practices to reduce cow health disorders during the periparturient periods (Burke et al., 2010, Roche et al., 2013).

The ability of cows to resist the establishment of diseases during the periparturient period is related to the efficiency of their immune system. The immune system consists of a variety of biological components and processes that serve to protect cows from the consequences of disease. The primary roles of the immune system are to prevent microbial invasion of the body, eliminate existing infections and other sources of cellular injury, and restore tissues to normal function. In animals, the immune system utilizes a multifaceted network of physical, cellular and soluble factors to facilitate defense against a diverse array of microbial challenges. This integrated system of defense mechanisms is highly regulated to maintain a delicate balance between the activation of immunity needed to prevent the establishment of disease and resolution of activity once the threat of invasion has passed. This paper will provide a brief overview of the immune system, describe how suboptimal immune responses can fail to prevent disease, and outline current strategies to optimize immune responses in food-animals during times of increased susceptibility to disease.

OVERVIEW OF THE IMMUNE SYSTEM

A properly functioning immune system should protect animals from a variety of pathogenic organisms including viruses, bacteria and parasites. To accomplish this task, the immune system utilizes a complex and dynamic network of defense mechanisms that can be conveniently separate into two distinct categories: innate immunity and adaptive or acquired immunity (Table 1). Within both innate and adaptive immunity, defense mechanisms can be classified further into physical barriers, cell-mediated immunity, and soluble or humoral immunity.

Table 1. Categories of the immune system

Innate Immunity	Adaptive Immunity
Non-specific or generic response	Antigen-specific response
Immediate following exposure (minutes)	Delayed following exposure (days)
Physical & mechanical barriers	No physical/mechanical barriers
Cellular and soluble factors	Cellular and soluble factors
No immune memory	Immunological memory
Inflammation	Antibody response (vaccines)

Innate Immunity

The innate immune system is the dominant host defense mechanism in most organisms. Innate immunity includes the nonspecific components of the immune system that can respond to infectious microbes in a generic manner. Components of innate immunity constitute the first line of defense against invading pathogens since they are already present or are activated quickly at the site of exposure. Depending on the efficiency of innate defenses, microbes may be eliminated within minutes to hours following invasion. This initial line of defense can be so rapid and efficient that there may be no noticeable changes in normal physiological functions of tissues as a consequence of the attempted microbial invasion. Because of its nonspecific nature, however, innate immune mechanisms are not augmented by repeated exposure to the same insult. Components of the innate immune system include physical and mechanical barriers, phagocytes, and various soluble mediators (Table 2). Physical and mechanical barriers are essential for preventing pathogens from entering the body. Some examples of surface barrier defenses that impede microbial invasion include the skin, tears, and mucus. Once pathogens are able to breach this initial line of defense, however, the cellular and soluble components of the innate immune response must act promptly to prevent the successful establishment of disease (Aitken et al., 2011).

Pattern recognition receptors play a critical role in innate immunity by sensing the presence of invading pathogens that successfully breach surface barrier defenses. Pattern recognition receptors can be expressed on cell surfaces, secreted, or expressed intracellularly and function to recognize a range of microbial factors associated with infectious pathogens. Collectively, this diverse array of conserved motifs unique to specific groups of microbes are referred to as pathogen-associated molecular patterns (PAMPs) and include, for example, lipopeptides of gram-positive bacteria and lipopolysaccharide of gram-negative bacteria (Jungi et al., 2011, Kumar et al., 2011). Some examples of pattern recognition receptors found in both immune and non-immune cells within animals that can differentiate a range of PAMPs are CD14, nucleotide-binding oligomerization domains (NOD), and the family of toll-like receptors (TLR) including TLR2, TLR4, TLR5, and TLR9 (Kumar et al., 2011). After binding to their ligand, pattern recognition receptors can initiate intracellular signaling cascades that result in initiation of innate immune responses or can facilitate antimicrobial activity directly (Aitken et al., 2011).

Table 2. Components of innate immunity

Factor	Main Functions
Physical barriers	Block & trap microbes (skin, tears, mucus)
Pattern Recognition Receptors	Surveillance and activation of innate immune responses
Complement	Bacteriolytic & facilitates phagocytosis
Cytokines	Pro-inflammatory & immunoregulatory
Oxylipids (prostaglandins, leukotrienes)	Pro-inflammatory & pro-resolving
Endothelial Cells	Regulates leukocyte migration & activation
Neutrophils	Phagocytosis and production of ROS
Macrophages	Phagocytosis; production of cytokines and oxylipids
Dendritic Cells	Phagocytosis; links innate & adaptive immunity
Natural Killer Cells	Targets and helps to eliminate infected host cells

Several endogenous soluble defenses of innate immunity can confront invading microbes that successfully enter the animal. Depending on the site of invasion, some of these soluble factors can be either pre-existing or induced through signaling cascades upon recognition of microbial PAMPs by pattern recognition receptors on host cells. Complement, for example, is a component of the innate defense system that consists of a collection of proteins present in serum and other body fluids. The proteins that comprise the complement system are synthesized mainly by hepatocytes, but other cellular sources include monocytes and tissue macrophages. Activation of the complement system can result in direct bactericidal activities from the deposition of pore-forming complexes (complement factors C3 and C5a) onto the surface of bacteria (Riollet et al., 2000). Other important biological functions of complement that contribute to early microbial killing include opsonizing bacteria and priming or activating host immune cells for phagocytosis and intracellular killing. Complement also is a potent chemoattractant for neutrophils and monocytes during the early stages of infection (Rainard, 2003).

Cytokines are an excellent example of soluble innate defenses that are induced following activation of pattern recognition receptors by PAMPs. For example, TLR activation leads to the activation of the NF□B signaling pathway where enhanced cytokine expression is a major response. The cytokine network consists of a diverse group of proteins produced by both immune and non-immune cells throughout the entire body and under diverse circumstances. The physiological and immunomodulatory capacity of the cytokine network is complex. Individual cytokines can interact with other cytokines synergistically, additively, or antagonistically on multiple cell targets. Several different cytokines can affect biological processes in the same way, as there is considerable functional redundancy within the cytokine network. Most cytokines have

very short half-lives, so their synthesis and function usually occurs in bursts of activity (Sordillo and Streicher, 2002). Cytokines are able to influence cellular functions through high affinity receptors for each cytokine located on host cells. Therefore, the activity of any responder cell is a function of not only the quantity and type of cytokine in the tissue microenvironment, but also the relative expression of cytokine receptors. With respect to innate immunity, cytokines exert their diverse effects by initiating the inflammatory response and facilitating the migration of leukocytes from blood into infected tissues following bacterial recognition by local cell populations. The pattern of cytokine expression by cells in the body will differ depending on the type of pathogen that elicits their response. In general, however, gram-negative bacteria initiate a greater magnitude of pro-inflammatory cytokine responses (i.e., IL1, IL6, IL8, and TNFα when compared to gram-positive bacteria that tend to express a weaker and slower cytokine response during the early stages of infection.

Oxylipids are a class of lipid mediators that contribute to innate immunity by regulating the onset, magnitude, and duration of the inflammatory response. Oxylipids are synthesized from polyunsaturated fatty acid substrates primarily found in the cellular membrane including the omega-6 linoleic and arachidonic acids or the omega-3 eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (Schmitz and Ecker, 2008). These fatty acid substrates are oxidized non-enzymatically by reactive oxygen species (ROS) or through different enzymatic pathways including the cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 pathways to produce a variety of oxylipids including prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), and lipoxins. Depending on the timing and magnitude of expression, certain oxylipids can either enhance or resolve the inflammatory response. Oxylipids can enhance inflammatory processes by increasing vascular permeability, leukocyte infiltration, localized edema, and fever. In response to microbial stimuli, for example, COX2 enzyme activity increases resulting in the biosynthesis of oxylipids with known vasoactive and proinflammatory properties such as PGE_2, $PGF_{2\alpha}$, and TXA_2. Non-steroidal anti-inflammatory drugs can inhibit PG and TX biosynthesis by targeting COX activity and are used to treat a variety of inflammatory-based diseases in food-animals. However, previous assumptions that all COX metabolites are solely responsible for propagating the inflammatory response is not supported by the current literature (Serhan et al., 2008). Whereas increased COX2 expression during the onset of inflammation is typified by PGE_2 production, the presence of COX-derived PGD_2 and 15d-PGJ_2 are associated with the resolution of inflammation (Prasad et al., 2008). More recent studies have led to the discovery of anti-inflammatory lipoxins (LX) that are produced from the metabolism of hydroperoxy fatty acid intermediates by the 5LOX pathway (Serhan et al., 2008). Omega-3 fatty acids also can be oxidized by COXs and LOXs to produce oxylipids with more anti-inflammatory or resolving properties (Schmitz and Ecker, 2008). For example, EPA is metabolized by 5LOX and modified forms of COX2 to produce E-series resolvins, whereas 15LOX converts DHA to the D-series resolvins, protectins, and the macrophage-specific maresin (Serhan and Chiang, 2008). During uncontrolled inflammation, a combination of exacerbated production of pro-inflammatory oxylipids and diminished production of anti-inflammatory oxylipids prevents full resolution and restoration of homeostasis of the affected tissues. Therefore, the balance between

production of pro- and anti-inflammatory oxylipids is one factor that determines the inflammatory phenotype of a cell/surrounding microenvironment (Mattmiller et al., 2013).

Inflammation

Inflammation is a critical component of the innate defense system that involves complex biological responses of both cellular and soluble factors following local tissue injury or trauma. The purpose of host inflammatory responses is to eliminate the source of tissue injury, restore immune homeostasis, and return tissues to normal function. The inflammatory cascade results not only in the escalation of local antimicrobial factors, but also in the increased movement of leukocytes and plasma components from the blood into infected tissues. The clinical signs of inflammatory include redness, heat, swelling, and pain(Mattmiller et al., 2013). These clinical symptoms can be explained by distinct changes in vascular endothelial responses. For example, redness, heat, and pain are caused by increased blood flow as a consequence of enlarged vascular diameter. Increased vascular permeability, resulting from the separation of tightly joined endothelial cells that line the blood vessel, leads to the exit of fluids and proteins from the blood and accumulated into tissues. These events account for the swelling or edema associated with inflammation.

In addition to changes in blood flow, the vascular endothelium also serves as a “gate-keeper” that regulates the movement of leukocytes from the blood vessels and into the affected tissues. Host cells express pattern molecule receptors that become activated by PAMPs and then release various inflammatory mediators such as cytokines and oxylipids that initiate the inflammatory cascade. These inflammatory mediators act directly on vascular endothelium to cause reductions in blood velocity with a concomitant increased expression of adhesion molecules on endothelial cells. Adhesion molecules on leukocytes attach to vascular adhesion molecules to facilitate the migration of leukocytes from the blood to the site of injury (Maddox et al., 1999, Hodgkinson et al., 2007). Neutrophils are the predominant cell type to undergo this extravasation process during the early stages of inflammation. Neutrophils first marginate and then adhere to the local endothelium near the site of infection. Cytokines, oxylipids, and other mediator molecules stimulate adherent neutrophils to move between endothelial cells and pass the basement membrane into the damaged tissue areas. The movement of neutrophils within the tissues is facilitated by chemotactic gradients created by inflammatory mediator molecules at the localized site of infection. Neutrophil migration can occur quickly and accumulate within affected tissues as soon as 30 to 60 minutes following injury (Summers et al., 2010).

Both newly recruited and pre-existing leukocyte populations act cooperatively to eliminate microbial pathogens. Macrophages localized within tissues respond to bacterial invasion by the release of immune-regulatory cytokines and oxylipids. Both macrophages and the newly recruited neutrophils also function to phagocytize and kill invading microbes. The process of phagocytosis involves the internalization of bacteria within phagosomes that contain bactericidal reactive oxygen species (ROS) and hydrolytic enzymes. The ROS are formed by respiratory burst activity that involves the

activation of NADPH oxidase and the subsequent production of superoxide radicals and hydrogen peroxide (Sordillo and Aitken, 2009). Myeloperoxides can further combine hydrogen peroxide with chloride to produce hypochlorite that is associated with bacterial activities. In addition to phagocytosis, neutrophils can kill bacteria through extracellular mechanisms. Activated neutrophils can form neutrophil extracellular traps (NETs) that consist of a web of fibers composed of chromatin and serine proteases that trap and kill bacteria. Studies suggest that NETs provide a highly concentrated foci of antibacterial substances that bind and kill bacteria independently of phagocytic uptake in the mammary gland (Lippolis et al., 2006, Grinberg et al., 2008). NETs also may serve as a physical barrier that prevents further spread of bacteria throughout the mammary gland.

Adaptive Immunity

The adaptive immune response is triggered when innate immune mechanisms fail to eliminate a pathogen. The adaptive immune response is characterized by the generation of antigen-specific lymphocytes and memory cells with the ability to recognize specific antigenic determinants of a pathogen. When host cells and tissues are re-exposed to the same antigen, a heightened state of immune reactivity occurs as a consequence of immunological memory and clonal expansion of antigen-specific effector cells. A memory immune response would be much faster, considerably stronger, longer lasting, and often more effective in clearing an invading pathogen when compared to a primary immune response. In contrast to the innate immune response, adaptive immunity can take days to develop because of the clonal expansion of B and T lymphocytes specific to the invading pathogen. An amazing feature of the immune system is the ability of a host to recognize and respond to billions of unique antigens that they may encounter. It also is important that an inappropriate specific immune response does not occur against the host's own antigens. For this reason, the immune system is able to distinguish self from non-self and selectively react to only foreign antigens. Genetically diverse, membrane bound proteins called major histocompatibility complex (MHC) molecules assist in this recognition. A specific immune response will only occur if antigens are combined with an MHC molecule on the surface of certain cells, a process referred to as antigen presentation (Sordillo and Aitken, 2011). The unique features of the adaptive immune response form the basis of vaccine strategies (Table 1).

Generation of effective specific immunity involves two types of cells, lymphocytes and antigen presenting cells. Lymphocytes recognize bacterial antigens through membrane receptors specific to the invading pathogen. These are the cells that mediate the defining attributes of adaptive immunity including specificity, diversity, memory, and self/non-self recognition. The T- and B-lymphocytes are two distinct subsets of lymphocytes which differ in function and protein products. The T lymphocytes can be further subdivided into □□ T-lymphocytes, which include CD4+ (T helper) and CD8+ (T cytotoxic) lymphocytes, and □□ T lymphocytes (Table 3). Depending on tissue location, the percentages of these cells can vary significantly (Sordillo and Streicher, 2002).

Table 3. Components of Adaptive Immunity

Factor	Main Functions
Major Histocompatibility Complex	Recognizes self from non-self
Dendritic Cells and Macrophages	Antigen presentation cells
T Lymphocytes	CD4+ Cells or T helper Cells (Th1, Th2, Th17, Treg); produce cytokines that regulate innate and adaptive immunity; immunoglobulin isotype switching
	CD8+ Cells or T cytotoxic (Tc); attacks and kills cells that express foreign antigens (virus-infected)
	□□□T Cells; prevalent at mucosal surfaces
B Lymphocytes	Antigen presentation; differentiate into antibody-producing plasma cells
Immunoglobulin (antibodies)	IgM is the largest and first produced; role in agglutination and complement activation
	IgG concentration is high in sera and is important for opsonization
	IgA is found at mucosal surfaces and has anti-viral function
	IgE is associated with allergic reactions and parasitic infections
	IgD non-secreted regulatory molecule

Effector functions of T lymphocytes include the production of cytokines that facilitate cell-mediated immunity by regulating the magnitude and duration of the immune response. The T helper lymphocytes (TH1, TH2, TH17, and Treg) produce cytokines in response to recognition of antigen-MHC complexes on antigen presenting cells (B lymphocytes and macrophages). When activated, T helper cells produce a variety of immunoregulatory cytokines. Through the ability to secrete certain cytokines, T helper cells play an important role in activating both T and B lymphocytes, macrophages, neutrophils, and various other cells that participate in the immune response. Differences in the particular pattern of cytokines produced by activated T helper cells results in different types of immune responses. For example, the cytokines IFN-γ and IL2 are thought to enhance some nonspecific cellular activities such as phagocytosis and intracellular killing (Sordillo and Aitken, 2011).

Antigen-specific B lymphocytes synthesize and secrete antibodies or immunoglobulin (Ig) that recognize and counteract specific microbial virulence factors. Immunoglobulins are produced by antigen-activated B-lymphocytes that subsequently proliferate and differentiate into antibody-secreting plasma cells. There are 4 classes of immunoglobulins that are known to influence host defense against infectious pathogens, namely IgM, IgG, IgA and IgM, which all differ in their physiochemical and biological

properties. For example, several Ig isotypes (IgG_1, IgG_2, and IgM) can act as opsonins to enhance phagocytosis by neutrophils and macrophages. In addition to its role in opsonization, IgM is efficient at complement fixation. Whereas IgA does not aid in bacterial opsonization, it does function in bacterial agglutination that can impede the ability of certain pathogens to spread throughout certain tissues. Another important role of IgA is its ability to neutralize some bacterial toxins. Clearly, both the concentration and isotype composition of Ig found in tissues can have a profound influence on the establishment of new infections.

PERIPARTURIENT IMMUNE DYSFUNCTION

The periparturient period is characterized as a time of dramatic changes in the efficiency of the bovine immune system. Numerous studies have documented changes in many aspects of both innate and adaptive immunity that can impact the susceptibility to new diseases in the transition cow (Aitken et al., 2011). A poorly functioning immune system can result in a number of adverse consequences. Not only are cows more likely to become infected when exposed to pathogenic organisms, but the severity of disease is also escalated. Dysfunctional inflammatory reactions that occur at both the systemic and local level, for example, are especially problematic because of the direct impact on disease pathogenesis in transition cows including metritis and mastitis. Derangements in inflammatory responses can consist of a hyporesponsive state characterized by delayed migration of functionally adequate neutrophils and other innate immune factors during the early stages of disease. Conversely, the lack of an appropriate balance between the initiation and resolution of inflammation can result in an overly robust or chronic inflammatory response characterized by extensive damage to host tissues. An excellent example of the consequence of a dysfunctional immune response is the severity and duration of mastitis in early lactation cows. Studies showed that the ability of mammary glands to promptly respond to *Escherichia coli* endotoxin during early lactation when compared to cows in mid-lactation (Grommers et al., 1989). The delayed migration of neutrophils and their reduced antimicrobial activity was suggested to be the cause of more severe coliform mastitis in the periparturient period when compared to later stages of lactation (Shuster et al., 1996) (Hill, 1981).

The underlying causes of dysfunctional inflammation during the transition period have been the subject of considerable research, with evidence to support a role for both endocrine and metabolic factors (Sordillo and Mavangira, 2014). For example, increases in several steroid hormones around the time of parturition are at least partially responsible for the altered function of neutrophils (Burton et al., 2005). Glucocorticoids are known to have potent immunosuppressive functions and plasma concentrations increase around the time of calving. A mechanism by which glucocorticoids can impair blood neutrophil function is by inducing a down-regulation of L-selectin and CD18 adhesion molecules needed for effective activation and migration to sites of tissue injury (Burton et al., 1995). Furthermore, changes in estradiol and progesterone concentrations just prior to calving were reported to have direct and indirect effects on the functional capabilities of immune cell populations (Roth et al., 1982). Changes in these steroid hormones do not overlap with the entire transition period; it is clear,

therefore, that other factors associated with the transition period also contribute to inflammatory dysfunction.

The role of negative energy balance (NEB) and changes in nutrient metabolism during the transition period has been implicated in the derangement of appropriate inflammatory responses (Sordillo and Raphael, 2013). In a series of elegant studies, for example, pregnant dairy cows were mastectomized to assess the impact of milk production and NEB on various immune parameters while still maintaining the endocrine changes associated with late pregnancy and parturition (Kimura et al., 1999, Kimura et al., 2002, Nonnecke et al., 2003). The mastectomized cows experienced only moderate increases in NEFA when compared with the cows with intact mammary glands during the periparturient period. Although immune function was compromised briefly around calving in mastectomized cows, lymphocyte and neutrophil functions were diminished longer in cows with mammary glands (Kimura et al., 1999, Nonnecke et al., 2003). These authors also reported a negative impact of lactation on the composition of peripheral blood leukocyte populations (Kimura et al., 2002). The major conclusion from these studies was that the act of parturition, with the associated changes in steroid hormone profiles, is not the major immunosuppressive factor in periparturient cows. Instead, the increased metabolic demands of early lactation was likely responsible for the adverse impact on immune cell populations. Indeed, there exist numerous studies that indicate how individual metabolic components may affect immune cell populations during the periparturient period including NEFA, betahydroxybutarate, changes in glucose and micronutrient availability (Sordillo and Mavangira, 2014). Changes in nutrient metabolism may form the common linkages between dysfunctional immune responses and the increased incidence of both metabolic and infectious diseases in periparturient dairy cattle.

BENEFITS OF OPTIMIZING IMMUNITY

Optimal host defenses against infectious pathogens occur when both innate and adaptive immune mechanisms are tightly regulated to effectively eliminate the injurious insults and return tissues to homeostasis. Rapid resolution of infections will eliminate bystander tissue damage and prevent any noticeable changes to the overall health and well being of food animals. Some pathogens, however, have intrinsic properties that make the efficient elimination by the immune system difficult, and attempts by local defenses to achieve control often results in significant tissue damage and production losses. Whereas antibiotic therapy remains the mainstay for the treatment of many infectious diseases, there is a need for alternative and adjunct therapeutic options that target host immune responses. The challenge is to selectively down-modulate harmful host responses without diminishing beneficial responses that facilitate elimination of invading pathogens. In contrast to antimicrobial drugs used to treat diseases in food animals, strategies that target host responses will minimize the risk of drug residues and the possibility of developing drug resistant bacteria.

The development of effective immunomodulatory strategies to control the establishment of disease can begin by understanding those conditions that compromise host defenses. Considerable research has established that the physiological stress during the periparturient period in dairy cattle is closely associated with dysfunction of several components of the innate and adaptive immune response (Sordillo et al., 1997). Certain hormones associated with parturition can explain some of the adverse effects on immunity during the periparturient period. Oxidative stress, however, can also compromise immune cells populations especially during times of enhanced metabolism or during inflammation (Sordillo and Aitken, 2009). Activation of the immune response requires energy and the immune system must compete for essential nutrients that are otherwise being used for growth, muscle accretion, and milk production. The development of uncontrolled acute or chronic inflammatory responses to infectious pathogens can not only cause bystander damage to host tissues, but also repartition nutrients resulting in dramatic reductions in animal growth and productivity (Aitken et al., 2011). Therefore, adjusting the nutrition of animals and improving management to reduce exposure to infectious pathogens can have a major impact on optimizing immunity in animals.

The development of innovative strategies that can enhance an otherwise impaired immune response could have a major impact on the incidence of diseases in food-producing animals. The challenge that confronts researchers now is to gain a better understanding of the complex interactions between the pathogenesis of bacteria, host responses needed to eliminate the pathogens from host tissues, and methods to enhance the immune potential of these factors before disease is established.

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